The Relationship between Serum Leptin and C - reactive protein in Iraqi Type 2 Diabetic Patients

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Abstract

Background: Higher serum levels of leptin and highly sensitive C-reactive protein (CRP) have been associated with type 2 diabetes mellitus (T2DM) as well as with obesity and increased risk of cardiovascular disease.

Aim of study: To test whether serum leptin has an association with CRP in type 2 diabetic patients independent of the influences of some factors that might contribute to evolution of T2DM and its complications.

Materials and methods: Eighty eight patients (44 males and 44 females) with T2DM were enrolled in this study. The following clinical characteristics were reported: Age, body mass index (BMI), blood pressure measurement, and family history of diabetes. Laboratory analyses included serum leptin, serum CRP, serum glucose assay, blood glycosylated hemoglobin (HbA_{1c}) assay, and lipid analysts which included serum cholesterol, serum triglycerides, and serum high density lipoprotein-cholesterol.

Results: The patients were categorized into a male group and a female group. Leptin showed a highly significant positive correlation with CRP ($r = 0.449$, $P<0.01$) as well as with BMI ($r =0.677$, $P<0.001$) in male patients. Leptin also showed a highly significant positive correlation with CRP ($r = 0.468$, $P<0.01$) as well as with BMI ($r = 0.479$, $P<0.001$) in female patients but in these patients CRP was also positively correlated with BMI ($r = 0.667$, $P<0.001$). The latter highly significant positive correlation was not detected in male patients. The correlation between leptin and CRP has remained significantly positive independent of age, duration of T2DM, fasting serum glucose and HbA_{1c} in both male and female group. The correlation, when studied independent of BMI, has remained significantly positive in male group but became non significant in female group.

Conclusion: Serum leptin level is associated with CRP level in type 2 diabetic patients. This association was independent of BMI in males and independent of many other variables that are associated with the evolution of T2DM in both males and females. This link may suggest a possible mechanism by which leptin may play a role in the development of diabetic complications such as cardiovascular disease.

Key words: Leptin, CRP, body mass index, type 2 diabetes mellitus

Introduction

Leptin is a 167 amino acid hormone that is almost exclusively produced by adipose tissue. It decreases appetite and increases energy expenditure, which consequently decreases adipose tissue mass and body weight [1, 2]. Leptin is coded by a specific gene called the obese (ob) gene that is expressed mainly in adipocytes. Obesity is a feature of ob/ob mice in which the leptin gene is deficient [3].

Although leptin is an anti-obesity factor, most obese human subjects have normal or high circulating leptin levels, i.e., human obesity is not a leptin-deficiency syndrome [4].

On the contrary, many obese people seem to be resistant to the hormonal effects of leptin and the basis for "leptin resistance" in human obesity is poorly understood [5].

High circulating leptin level has been shown to be associated with subsequent development of type 2 diabetes mellitus (T2DM) [6] for which obesity is a major risk factor [7]. The underlying mechanism for these associations between higher leptin level, obesity and T2DM have not been completely explained but higher leptin level has been linked with obesity-induced inflammation that by itself may contribute to T2DM [8, 9]. In many studies the best correlations between measures of obesity and markers of inflammatory response were related to circulating levels of C-reactive protein (CRP) [10]. High serum level of (CRP) has also been reported to be associated with incident [11] or established T2DM [12] and the factors that are responsible for elevation have not yet been fully clarified. Thus both leptin and CRP have been associated with obesity [4, 13], inflammation [13] and T2DM [6,11].

Additionally, both have also been linked to increased cardiovascular risk [14,15].

There is little information on an interaction between leptin and CRP, particularly in T2DM. We tested whether serum leptin has an association with CRP independent of the influences of some factors that might contribute to evolution of T2DM and its complications.

Materials and Methods:

The study was conducted in the National Diabetes Center, University of Al–Mustansiriya, and Baghdad -Iraq. Eighty eight patients with type 2 diabetes mellitus were enrolled in the study (44 males and 44 females). Their ages were in the range of (31-70) years and the duration of their disease has ranged from (2-25) years.

Medical examination:

Medical history was taken by personal interviewing. Exclusion was made for those who had a concurrent acute illness or another major systemic disease except hypertension. Also patients who were taking lipid lowering agents or were smokers were excluded as well as those who had evidence of ischemic heart disease or ischemic stroke. Blood pressure measurements higher than 140/90 or the presence of hypertension on treatment was reported as hypertensive [16]. The presence of diabetes in a first

degree relative was taken as an evidence of having family history of diabetes.

Blood glucose assay:

Glucose was measured by enzymatic method using kits supplied by Biocon Company, Germany.

HbA_{1c} assay:

HbA_{1C} was measured by Bio-Rad VARIANT Hemoglobin Testing System (Bio–Rad Laboratories, Inc., Hercules, CA 94547, USA).

Lipid analytes:

Serum total cholesterol and serum triglycerides (TG) level were determined by totally enzymatic methods. Estimation of serum high density lipoprotein cholesterol (HDL-C) was done by precipitation with phosphotungstate-$MgCl_2$ solution followed by enzymatic determination of cholesterol in the supernatant [17]. TG level > 150 mg / dl (1.70 mmol/L) was considered abnormal. HDL-C level < 40 mg/dl in males (1.04 mmol/L) and < 45 in females (1.17 mmol/L) was considered abnormal. The patient was classified as dyslipidemic if both TG and HDL-C levels were abnormal [18].

Statistical analysis:

Statistical analysis was done by using Student's t-test for the significance of difference between two mean values and simple linear correlation was used for determination of correlation between two quantitative parameters for different groups. A probability value ($p<0.05$) was considered to be statistically significant.

Results:

The patients were categorized into a male group and a female group. There were no significant differences between the two groups in regard to age and duration of disease (Table 1). The mean BMI values of both groups were in the range of obesity and the mean value in female group was higher than in male group although the difference was statistically non significant (32.32±6.78 and 30.34±4.43 kg/m^2 respectively)

In regard to biochemical analytes, fasting serum glucose, glycosylated hemoglobin (HbA1c), serum TG and HDL-C mean levels showed no significant differences but TC mean level was higher in females than in males and the difference was statistically significant (Table 2). Serum CRP mean level showed no significant difference although it was somewhat higher in females than in males (1.39± 1.43 in males and 1.75 ±1.24 mg / L in females respectively) while serum leptin mean level was higher in females than in males (14.72 ±11.34 and 6.82± 4.87 ng / ml, respectively) and the difference was statistically highly significant ($P < 0.01$).

Correlations between leptin, CRP, and some clinical and biochemical characteristics are presented in (Table 3) for male patients and in (Table 4) for female patients. Leptin showed a highly significant positive correlation with CRP ($r = 0.449$, $P<0.01$) as well as with BMI ($r =0.677$, $P<0.001$) in male patients. Leptin also showed a highly significant positive correlation with CRP ($r = 0.468$, $P<0.001$) as well as with BMI ($r = 0.479$, $P<0.001$) in female patients but in these patients CRP was also positively correlated with BMI ($r = 0.667$, $P<0.001$). The latter highly significant positive correlation was not detected in male patients.

To adjust for the effect of potential confounders, the correlation between leptin and CRP was studied independently of certain clinical or biochemical characteristics in male and female patients (Table 5).

The correlation remained significantly positive independent of age, duration of diabetes, fasting serum glucose and HbA_{1c} in both male and female group. The correlation between leptin and CRP, when studied independent of BMI, remained significantly positive in male group but became non significant in female group (Table 5).

After the analysis of the correlations and to further analyze the relations of the prominent findings in the study, the mean values of BMI, serum leptin and CRP were compared in the presence or absence of a selected clinical characteristic in both male and female group (table 6 and table 7 respectively).

These clinical characteristics included family history of diabetes, presence of hypertension or presence of dyslipidemia. In male group, mean serum leptin level was significantly higher in patients who had had dyslipidemia (9.01±4.47 vs. 5.32±4.63, $P<0.05$). In female group, mean serum leptin level was significantly higher in patients who had had hypertension (17.51± 12.14 vs. 10.29±8.48, $P<0.05$).

Table 1: Clinical characteristics and body mass index (BMI) of the study patients

Characteristic	Groups	Mean ± SD (N=44)	P- value
Age (year)	Male group	54.64± 11.99	NS
	Female group	55.98± 8.07	
Duration of DM (year)	Male group	8.05± 6.61	NS
	Female group	8.59± 6.67	
BMI (kg/m^2)	Male group	30.34± 4.43	NS
	Female group	32.32± 6.78	

Table 2: Biochemical analytes of the study patients

Analyte	Groups	Mean ± SD (N=44)	P- value
Fasting serum glucose (mmol / L)	Male group	9.58 ± 3.83	NS
	Female group	9.48 ± 2.91	
HbA_{1c} (%)	Male group	8.25± 1.71	NS
	Female group	8.91± 1.70	
TC (mmol/L)	Male group	5.29 ± 0.29	P<0.01
	Female group	5.76 ± 1.22	
TG (mmol/L)	Male group	1.76 ± 0.75	NS
	Female group	1.64 ± 0.65	
HDL-C (mmol/L)	Male group	1.18 ± 0.10	NS
	Female group	1.16 ± 0.07	
CRP (mg/L)	Male group	1.39± 1.43	NS
	Female group	1.75± 1.24	
Leptin (ng/ml)	Male group	6.82± 4.87	P<0.01
	Female group	14.72±11.34	

Table 3: Correlations between leptin, CRP, & some clinical & biochemical characteristics of male patients

	Duration of DM	BMI	Fasting serum glucose	HbA_1c	CRP	Leptin
Age	r = 0.469** P<0.001	r = 0.066 NS	r = -0.055 NS	r =-0.148 NS	r = -0.230 NS	r= 0.120 NS
Duration of DM	.	r = 0.037 NS	r = 0.253 NS	r = 0.078 NS	r = -0.090 NS	r= 0.186 NS
BMI			r = 0.041 NS	r = 0.123 NS	r= 0.231 NS	r= 0.677** P<0.001
Fasting serum glucose				r= 0.717** P<0.001	r= -0.074 NS	r= -0.113 NS
HbA_{1c}					r= -0.046 NS	r = -0.189 NS
CRP						r= 0.449** P<0.01

NS (non significant)
P<0.01 (highly significant)

Table 4: Correlations between Leptin, CRP, and some clinical and biochemical characteristics of female patients

	Duration of DM	BMI	Fasting serum glucose	HbA_{1c}	CRP	Leptin
Age	r =0.395** P<0.01	r = -0.183 NS	r = 0.067 NS	r = 0.219 NS	r = 0.009 NS	r = 0.187 NS
Duration of DM		r= -0.104 NS	r = 0.489** P<0.001	r = 0.498** P<0.001	r = 0.095 NS	r = 0.251 NS
BMI			r = -0.172 NS	r = -0.117 NS	r = 0.667** P<0.001	r = 0.479** P<0.001
Fasting serum glucose				r = 0.627** P<0.001	r = -0.130 NS	r = 0.163 NS
HbA_{1c}					r = -0.053 NS	r = -0.079 NS
CRP						r = 0.468** P<0.001

Table 5: Correlation between leptin and hs CRP in study patients independent of some clinical and biochemical characteristics

Characteristic	Correlation between CRP & leptin independent of a certain characteristic			
	Male		Female	
	R	P- value	R	P- value
Age	0.493	P<0.001	0.475	P<0.001
Duration of DM	0.476	P<0.001	0.461	P<0.01
BMI	0.408	P<0.01	0.227	NS
Fasting Serum glucose	0.444	P<0.01	0.500	P<0.001
HbA1c	0.449	P<0.01	0.466	P<0.01
Age, Duration of DM, BMI, fasting serum glu-	0.448	P<0.01	0.182	NS

Table 6: Values of body mass index, serum leptin and C-reactive protein compared according to the presence or absence of a selected clinical characteristic in male patients

Clinical characteristic (N=44)	Groups	BMI (kg/m^2) Mean ± SD	CRP (mg/L) Mean ± SD	Leptin (ng/ml) Mean± SD
Family history of DM	Positive (N=32)	30.43± 4.31 NS	1.41±1.54 NS	6.71±4.40 NS
	Negative (N=12)	30.08±4.93	1.35±1.15	7.14±6.17
Hypertension	Positive (N=27)	29.98±5.00 NS	1.26±0.92 NS	7.53±5.43 NS
	Negative (N=17)	31.02±3.08	1.65±2.11	5.46±3.27
Dyslipidemia	Positive (N=18)	31.50±5.21 NS	1.62±0.94 NS	9.01±4.47 P<0.05
	Negative (N=26)	29.53±3.70	1.23±1.68	5.32±4.63

Positive: Presence of the selected clinical characteristic
Negative: Absence of the selected clinical characteristic

Table 7: Values of body mass index, serum leptin and C-reactive protein compared according to the presence or absence of a selected clinical characteristic in female patients

Clinical characteristic (N=44)	Groups	BMI (kg/m^2) Mean ± SD	CRP (mg/L) Mean ± SD	Leptin (ng/ml) Mean ±SD
Family history of DM	Positive (N=32)	33.47± 7.20 NS	1.78±1.32 NS	16.30±12.05 NS
	Negative (N=12)	29.26± 4.40	1.67±1.04	10.51±8.17
Hypertension	Positive (N=27)	32.64± 6.94 NS	1.81± 1.16 NS	17.51± 12.14 P<0.05
	Negative (N=17)	31.82± 6.70	1.64±1.38	10.29±8.48
Dyslipidemia	Positive (N=31)	31.31±5.18 NS	1.44±0.77 P<0.01	14.98±11.50 NS
	Negative (N=13)	34.73±9.42	2.45±1.79	14.11±11.38

Positive: Presence of the selected clinical characteristic
Negative: Absence of the selected clinical characteristic

Discussion:

The presence of an association between leptin and CRP has already been described in many studies on healthy subjects [19-21] but the mechanism linking leptin and CRP is not clear. Our study has shown a highly significant positive correlation between serum leptin and CRP in type2 diabetic males and females considered separately. Adipose tissue is the main source of circulating leptin [22].CRP is synthesized by the liver under the regulation of the proinflammatory

cytokines, primarily interleukin-6 (IL-6) and other cytokines such as IL-1 and tumor necrosis factor-α [23]. Inflammatory cells such as monocytes and macrophages within the adipose tissue may contribute to the synthesis of IL-6 [24]. Therefore, the presence of an inflammation in adipose tissue may be a source of such cytokines [4,13,25].

Adipocytes are also an important source of circulating IL-6 [4]. Hence, adipose tissue, by serving as a common source for both leptin and inflammatory cytokines may explain in part the interaction between CRP levels and leptin. An another way may be that the IL-6, and other cytokines, may act directly on fat cells to increase leptin secretion in the settings of inflammation [26] ,further supporting adipose tissue as a potential common pathway contributing to the leptin – CRP interaction.

The significant relationship between leptin and CRP in the present study was independent of the measure of adiposity. Moreover, the highly significant positive correlation between CRP and BMI was not detected in our male group. Furthermore, in a study on subjects with BMI <25 kg/m2, CRP was significantly linked to leptin but not to BMI [21]. It may thus be said that the presence of an inflammation in adipose tissue may not be the important source for the increased level of these cytokines in all situations.

Another possibility may be that leptin itself may alter CRP levels. Leptin has been shown to induce the expression of CRP in vascular endothelial cells [27] and in human primary hepatocyte [28].

Moreover, evidence was presented for human CRP inhibiting the binding of leptin to its receptors in vitro and therefore an interaction between CRP and leptin was proposed as a mechanism contributing to leptin resistance.

An approach that disrupts the interaction between leptin and CRP, such as by an anti-dot to CRP, to solve the problem of leptin resistance has also been suggested [28] .However, the suggestion needs to be studied further [29].

The present study has detected a highly significant positive correlation between CRP and BMI in female group but not in male group. Sex difference in the prediction of T2DM by inflammatory markers has already been reported [30]. Moreover, CRP and BMI have been found to affect the relation between leptin and indices of atherosclerosis, as an inflammatory disease, in men and women differently. Reilly et al, on a sample comprised mainly of T2DM men found that the relation remained significant even after adjustment for BMI and for CRP [31] . Iribarren ,C et al , on a sample comprised mostly of women found that the relation was abolished after adjustment for measures of obesity or if CRP was kept constant by multivariable regression [32].

Our finding of an association between higher leptin level and hypertension in women and dyslipidemia in men is consistent with the findings in many other studies. In a study on the association between hyperleptinemia and metabolic syndrome in an urban Korean community, leptin was clustered as an obesity/hypertension factor in women and as insulin resistance/dyslipidemia factor in men [33] .This was also reported by other studies [34] and this gender difference was interpreted to reflect different roles of central adiposity on metabolic abnormalities [33] . In an another study, serum leptin level was found to be markedly high in obese hypertensive women [35], such women were characterized by hypercholesterolemia but no significant dyslipidemia and this is consistent with our results. The association between high BMI or obesity and higher serum cholesterol level has been reported by other studies [36].

The findings of our study have many implications. If leptin does indeed induce CRP, this may be one of the mechanisms whereby leptin would increase diabetic complications such as cardiovascular disease (CVD). The conditions in which both leptin and CRP may be increased simultaneously, such as obesity and T2DM are needed to be investigated for a possible additive or perhaps synergistic increases in cardiovascular risk.

The sex difference should also be further investigated knowing that normal ranges of leptin and CRP are higher in females than males while the risk of CVD in males is known to be higher than in females.

In summary, serum leptin is associated with CRP in type 2 diabetic patients. This association was independent of body mass index in males and independent of many other variables that are associated with the evolution of T2DM in both males and females. This link between a metabolic and an inflammatory mediator in T2DM may suggest a possible pathophysiological mechanism or mechanisms (depending on the gender) by which leptin may play a role in the development of diabetic complications such as CVD.

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